This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. (Original) A method of improving a concentration detection limit for an ampholytic analyte in an isoelectric focusing system comprising the steps of:

providing an isoelectric focusing system having a separation compartment disposed between an anode compartment and a cathode compartment;

providing a solution containing an ampholytic analyte and a mixture of carrier ampholytes;

providing at least one of the options selected from the group consisting of option one and option two, wherein option one uses one or more auxiliary compartments disposed between at least one of the anode compartment and the separation compartment or the cathode compartment and the separation compartment, and option two uses one or more auxiliary agents mixed with the solution containing the ampholytic sample component;

filling the anode compartment with an acidic solution and the cathode compartment with a basic solution;

filling the other compartments with the solution containing the ampholytic analyte;

applying a potential between an anode located in the anode compartment and a cathode located in the cathode compartment and effecting an isoelectric focusing of the ampholytic analyte into the separation compartment; and

detecting the focused ampholytic analyte in the separation compartment at its increased concentration over that provided by isoelectric focusing without the use of option one or option two.

2. (Original) A method of improving a concentration detection limit for an ampholytic analyte in an isoelectric focusing system and eliminating a deformation of a pH gradient in the isoelectric focusing analysis of a salt-laden sample containing an ampholytic analyte comprising the steps of:

providing an isoelectric focusing system having a separation compartment disposed between an anode compartment and a cathode compartment;

providing one or more auxiliary compartments disposed between at least one of the anode compartment and the separation compartment or the cathode compartment and the separation compartment;

adding a mixture of carrier ampholytes and a first amount of one or more auxiliary agents to the salt-laden sample solution containing the ampholytic analyte;

filling the anode compartment with an acidic solution and the cathode compartment with a basic solution;

filling the other compartments with the solution containing the ampholytic analyte;

applying a potential between an anode located in the anode compartment and a cathode located in the cathode compartment and effecting a first isoelectric focusing of the ampholytic analyte into the separation compartment;

detecting at a first focusing position in the separation compartment the focused ampholytic analyte;

adjusting the first amount of the one or more auxiliary agents added to the salt-laden sample solution containing the ampholytic analyte to a second amount and effecting a second isoelectric focusing of the ampholytic analyte into the separation compartment; and

detecting at a desired second focusing position in the separation compartment the focused ampholytic analyte at its increased concentration over that provided in an isoelectric focusing without the use of an auxiliary compartment or an auxiliary agent.

3. (Original) A method according to Claim 1 or 2, wherein the isoelectric focusing system is a capillary isoelectric focusing system.

4. (Original) A method according to Claim 1 or 2, wherein the isoelectric focusing system is an imaging capillary isoelectric focusing system.

5. (Original) A method according to Claim 1 or 2, wherein the isoelectric focusing system is a chip-based isoelectric focusing system.

6. (Original) A method according to Claim 1 or 2, wherein the isoelectric focusing system is a chip-based imaging isoelectric focusing system.

7. (Original) A method according to Claim 1 or 2, wherein the auxiliary compartment and the adjacent electrode compartment are separated by an anti-convective, ion-permeable barrier that substantially eliminates convective mixing between the contents of the auxiliary compartment and the adjacent electrode compartment.

8. (Original) A method according to Claim 1 or 2, wherein the auxiliary compartment and the adjacent electrode compartment are separated by an anti-convective, ion-permeable membrane

that substantially eliminates convective mixing between the contents of the auxiliary compartment and the adjacent electrode compartment.

9. (Original) A method according to Claim 1 or 2, wherein any auxiliary agent used is selected from a group consisting of subgroups of strong electrolytes, weak electrolytes, and ampholytes.

10. (Original) A method according to Claim 1 or 2, wherein the multiple auxiliary agents used are selected to belong to the same or different subgroups of strong electrolytes, weak electrolytes, and ampholytes.

11. (Original) A method according to Claim 1 or 2, wherein the difference between the pI value of the ampholytic auxiliary agent and its nearest pKa value is less than 2.

12. (Original) A method according to Claim 1 or 2, wherein the difference between the pI value of the ampholytic auxiliary agent and its nearest pKa value is less than 1.

13. (Original) A method according to Claim 1 or 2, wherein the difference between the pI value of the ampholytic auxiliary agent and its nearest pKa value is less than 0.75.

14. (Original) A method according to Claim 1 or 2, wherein the pI value of one or more of the ampholytic auxiliary agents is lower than the pI value of the most acidic ampholytic analyte of interest or higher than the pI value of the most basic ampholytic analyte of interest.

15. (Original) A method according to Claim 1 or 2, wherein one or more of the auxiliary agents absorb light at a selected detection wavelength.

16. (Original) A method according to Claim 1 or 2, wherein one or more of the auxiliary agents fluoresce.

17. (Original) A method according to Claim 1 or 2, wherein one or more of the ampholytic auxiliary agents are selected from a group consisting of cysteic acid, N,N-dimethyliminodiacetic acid, N-methyliminodiacetic acid, iminodiacetic acid, benzeneiminodiacetic acid, aspartic acid, glutamic acid, ornithine, lysine, terbutaline, tyramine, arginine.

18. (Currently Amended) A method according to Claim 1 or 2, wherein any member of a group consisting of hydronium, lithium, sodium, potassium, tetramethylammonium, tetraethylammonium, tetrapropylammonium, tetrabutylammonium, benzyltrimethylammonium, benzyltriethylammonium, benzyltripropylammonium, benzyltributylammonium, alkoxybenzyltrimethylammonium ions can be used as a non-hydrolyzing cation for ~~the~~ a strong or weak electrolyte auxiliary agent, and any member of a group consisting of hydroxide, chloride, bromide, iodide, sulfate, nitrate, methanesulfonate, ethanesulfonate, benzenesulfonate, toluenesulfonate, naphthalenesulfonate, benzenedisulfonate, naphthalenedisulfonate and alkoxybenzenesulfonate ions can be used as a non-hydrolyzing anion for ~~the~~ a strong or weak electrolyte auxiliary agent.

19. (Currently Amended) A method according to Claim 1 or 2, wherein any member of a group

consisting of ammonium, monoalkylammonium, dialkylammonium, trialkylammonium, arylalkylammonium, alkoxyarylalkylammonium ions can be used as a hydrolyzing cation for ~~the~~ a weak electrolyte auxiliary agent, and any member of a group consisting of alkylcarboxylate, arylcarboxylate, alkylarylcarboxylate, alkoxyarylcarboxylate, phenolate and alkoxyphenolate ions can be used as a hydrolyzing anion for ~~the~~ a weak electrolyte auxiliary agent.

20. (Original) A method according to Claim 1 or 2, wherein one or more solubilizer selected from a group consisting of non-electrolytes and zwitterions is additionally added to the sample solution to increase the solubility of the ampholytic analyte.

21. (Original) A method according to Claim 1 or 2, wherein one or more complexing agent selected from a group consisting of non-electrolytes and zwitterions is additionally added to the sample solution to improve the isoelectric focusing separation of the ampholytic analyte.

22. (Original) An apparatus comprising:

a separation compartment disposed between an anode compartment and a cathode compartment;

an anode disposed in the anode compartment and a cathode disposed in the cathode compartment;

one or more auxiliary compartments disposed between the anode compartment and the separation compartment or the cathode compartment and the separation compartment;

a means of filling the anode compartment with an acidic solution and the cathode compartment with a basic solution;

a means of filling the rest of the compartments with a solution that contains an ampholytic analyte, and one or more components selected from a group comprising a mixture of carrier ampholytes, strong electrolyte auxiliary agents, weak electrolyte auxiliary agents, and ampholytic auxiliary agents;

a means of applying a separation potential to the anode and the cathode and effecting an isoelectric focusing of the ampholytic analyte into the separation compartment; and

a means of detecting the focused ampholytic analyte in the separation compartment at its increased concentration over that provided by isoelectric focusing without the use of any auxiliary compartment and auxiliary agent.

23. (Original) An apparatus comprising:

a separation compartment disposed between an anode compartment and a cathode compartment;

an anode disposed in the anode compartment and a cathode disposed in the cathode compartment;

one or more auxiliary compartments disposed between the anode compartment and the separation compartment or the cathode compartment and the separation compartment;

a means of filling the anode compartment with an acidic solution and the cathode compartment with a basic solution;

a means of filling the rest of the compartments with a solution that contains an ampholytic analyte present in a salt-laden sample and a first amount of one or more components selected from a group comprising a mixture of carrier ampholytes, strong electrolyte auxiliary agents, weak electrolyte auxiliary agents, and ampholytic auxiliary agents;

a means of applying a separation potential to the anode and the cathode and effecting a first isoelectric focusing of the ampholytic analyte into the separation compartment;

a means of detecting at a first focusing position in the separation compartment the focused ampholytic analyte at its increased concentration;

a means of adjusting in the ampholytic analyte containing solution the first amount of the one or more components selected from the group comprising a mixture of carrier ampholytes, strong electrolyte auxiliary agents, weak electrolyte auxiliary agents, and ampholytic auxiliary agents to a second amount and effecting a second isoelectric focusing of the ampholytic analyte; and

a means of detecting at a desired second focusing position in the separation compartment the ampholytic analyte at its increased concentration over that provided by isoelectric focusing without the use of any auxiliary compartment and auxiliary agent.

24. (Currently Amended) An apparatus according to Claim 22 or 23, wherein there is one auxiliary compartment disposed between the anode compartment and the separation compartment and another auxiliary compartment disposed between the separation compartment and the cathode compartment;.

25. (Original) An apparatus according to Claim 22 or 23, wherein the separation compartment is part of a capillary isoelectric focusing system.

26. (Original) An apparatus according to Claim 22 or 23, wherein the separation compartment is part of an imaging capillary isoelectric focusing system.

27. (Original) An apparatus according to Claim 22 or 23, wherein the separation compartment is part of an isoelectric focusing system.

28. (Original) An apparatus according to Claim 22 or 23, wherein the separation compartment is part of an imaging isoelectric focusing system.

29. (Original) An apparatus according to Claim 22 or 23, additionally including an anti-convective, ion-permeable barrier between the auxiliary compartment and the adjacent electrode compartment that substantially eliminates convective mixing between the contents of the auxiliary compartment and the adjacent electrode compartment.

30. (Original) An apparatus according to Claim 22 or 23, additionally including an anti-convective, ion-permeable membrane between the auxiliary compartment and the adjacent electrode compartment that substantially eliminates convective mixing between the contents of the auxiliary compartment and the adjacent electrode compartment.

31. (Cancelled)

32. (Cancelled)

33. (Cancelled)

34. (Cancelled)

35. (Currently Amended) A method of improving a concentration detection limit for an ampholytic analyte in an isoelectric focusing system, comprising the steps of:

providing an isoelectric focusing system including a separation compartment disposed between an anode compartment having an anode therein and a cathode compartment having a cathode therein;

providing a solution containing an ampholytic analyte and a mixture of carrier ampholytes;

mixing at least one auxiliary agent with the solution containing the ampholytic analyte and mixture of carrier ~~amphlytes~~ ampholytes;

filling the anode compartment with an acidic solution and the cathode compartment with a basic solution;

filling the separation compartment with the solution containing the ampholytic analyte, mixture of carrier ~~amphlytes~~ ampholytes, and at least one auxiliary agent;

applying a potential between the anode located in the anode compartment and the cathode located in the cathode compartment to effect an isoelectric focusing of the ampholytic analyte in the separation compartment; and

detecting the focused ampholytic analyte in the separation compartment at its increased concentration over that provided by isoelectric focusing without the use of the at least one auxiliary agent.

36. (Currently Amended) A method of improving the concentration detection limits in an

isoelectric focusing system according to Claim 35, additionally including the step of adding at least one auxiliary compartment disposed between at least one of the anode compartment and the separation compartment and the cathode compartment and the separation compartment, and filling, along with the separation compartment, the at least one auxiliary compartment with the solution containing the ampholytic analyte and mixture of carrier ~~amphlytes~~ ampholytes.

37. (Currently Amended) A method of improving a concentration detection limit for an ampholytic analyte in an isoelectric focusing system, comprising the steps of:

providing an isoelectric focusing system including a separation compartment disposed between an anode compartment having an anode therein and a cathode compartment having a cathode therein;

providing a solution containing an ampholytic analyte and a mixture of carrier ampholytes;

providing at least one auxiliary compartment disposed between at least one of the anode compartment and the separation compartment and the cathode compartment and the separation compartment;

filling the anode compartment with an acidic solution and the cathode compartment with a basic solution;

filling the separation compartment and the at least one auxiliary compartment with the solution containing the ampholytic analyte and mixture of carrier ~~amphlytes~~ ampholytes;

applying a potential between the anode located in the anode compartment and the cathode located in the cathode compartment to effect an isoelectric focusing of the ampholytic analyte in the separation compartment; and

detecting the focused ampholytic analyte in the separation compartment at its increased concentration over that provided by isoelectric focusing without the use of the at least one auxiliary compartment.

Drawing Amendments

The attached sheet of drawings includes a change to FIG. 5. This sheet, which includes FIGS. 5 and 6, replaces the original sheet containing FIGS. 5 and 6.